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Genotypic differences in behavioural entropy: unpredictable genotypes are composed of unpredictable individuals

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Intragenotypic variability (IGV) occurs when individuals with the same genotype, reared in the same environment and then tested under the same conditions, express different trait values. Game theoretical and bet-hedging models have suggested two ways that a single genotype might generate variable behaviour when behavioural variation is discrete rather than continuous: behavioural polyphenism (a genotype produces different types of individuals, each of which consistently expresses a different type of behaviour) or stochastic variability (a genotype produces one type of individual that randomly expresses different types of behaviour over time). We first demonstrated significant differences across 14 natural genotypes of male *Drosophila melanogaster* in the variability (as measured by entropy) of their microhabitat choice in an experiment in which each fly was allowed free access to four different types of habitat. We then tested four hypotheses about ways that within-individual variability might contribute to differences across genotypes in the variability of microhabitat choice. There was no empirical support for three hypotheses (behavioural polymorphism, consistent choice, or time-based choice), nor could our results be attributed to genotypic differences in activity levels. The stochastic variability hypothesis accurately predicted the slope and the intercept of the relationship across genotypes between entropy at the individual level and entropy at the genotype level. However, our initial version of the stochastic model slightly but significantly overestimated the values of individual entropy for each genotype, pointing to specific assumptions of this model that might need to be adjusted in future studies of the IGV of microhabitat choice. This is among a handful of recent studies to document genotypic differences in behavioural IGV, and the first to explore ways that genotypic differences in within-individual variability might contribute to differences among genotypes in the predictability of their behaviour.

Behaviour is among the most labile of traits, and for many years biologists have studied factors that contribute to individual differences in behaviour. Major sources of behavioural variability include genes, age, sex, experiences prior to a behavioural assay and conditions during a behavioural assay (see Clark & Ehlinger 1987 for a review of the early literature; also see Sih et al. 2004; Dingemanse et al. 2010; Stamps & Groothuis 2010a, b; Groothuis & Trillmich 2011; Walker & Mason 2011). However, even if researchers carefully control for variation in all of these factors, experimental subjects do not always behave the same way. For instance, despite decades of attempts to standardize the genomes, rearing and testing environments of laboratory mice and rats, considerable variability remains in the behaviour of virtually isogenic strains (Lewejohann et al. 2011). Similarly, individuals from the same inbred line of *Drosophila melanogaster* may express different types of behaviour or make different choices, even if they are raised under highly standardized conditions, and then tested at the same age using a stringently controlled behavioural assay (e.g. Miller et al. 2011; Del Pino et al. 2012; Kain et al. 2012).

Here, we use the term intragenotypic variability, or IGV, to denote the variability of isogenic subjects, all of which have been reared under the same, carefully controlled conditions, and then measured or tested at the same age in the same context (where 'context' here indicates the external stimuli that surround an individual when its trait values are measured; see Stamps & Groothuis 2010a). Several recent studies have shown that IGV in behaviour can vary across different genotypes from the same species (Perry et al. 2010; Miller et al. 2011; Schuett et al. 2011; Kain et al. 2012). The notion that genotypes might differ with respect

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to the variability of their phenotypes is not new: morphologists have for many years documented genotypic differences in phenotypic variability, and gone on to consider the proximate mechanisms that contribute to these differences. For instance, in *D. melanogaster*, significant differences among genotypes in the variability of traits such as sternopleural bristle number (Dworkin 2005) and wing shape (Breuker et al. 2006) have been used to estimate genotypic differences in canalization, where canalization indicates the sensitivity of a genotype to environmental perturbations during ontogeny (Willmore et al. 2007). More recently, geneticists have begun to investigate loci that contribute to heterogeneity among genotypes in the variability of morphological, life history and behavioural traits (e.g. Kain et al. 2012; Shen et al. 2012).

However, there is one key difference between IGV for behaviour and IGV for traits that are temporally stable within individuals. In the case of temporally stable traits (i.e. most morphological and many life history traits), each individual need only be measured once, and the IGV of each genotype is equivalent to the interindividual variability of that genotype. However, behaviour can vary across time within as well as across individuals, as a result of many different proximate mechanisms. Some of these processes generate systematic temporal changes in behaviour over time (e.g. circadian rhythms, which affect the time of day that animals respond to particular stimuli or express particular types of behaviour, and gradual increases or decreases in behaviour over time in response to initially novel stimuli, such as habituation, sensitization, acclimation). Other processes generate short-term, pseudorandom temporal fluctuations in behaviour. In humans, this type of stochastic variability in behaviour within individuals has been termed intraindividual variability or IIV (Nesselroade 1991; Ram & Gerstorf 2009). Recent studies of animals indicate that IIV can significantly vary across individuals (Stamps et al. 2012); such individual differences in IIV are typically attributed to differences among individuals in fluctuations in neuronal or hormonal factors that affect the expression of behaviour (Brembs 2011). Hence, in the case of behaviour, IGV could be due to different types of within-individual variability, to interindividual variability, or to some combination of these.

Intriguingly, theoreticians have for many years not only assumed that IGV in behaviour varies among genotypes, but also identified two ways that a given genotype might generate variable phenotypes. In behavioural ecology, classic game theory models discriminate between genotypes with fixed strategies (invariant behaviour) and genotypes with mixed strategies (variable behaviour) (Maynard Smith & Price 1973; Maynard Smith 1982). In addition, they differentiate mixed strategies based on multiple behavioural phenotypes, each of which consistently expresses the same behaviour (e.g. 40% of the individuals with a given genotype always play hawk, while the remaining 60% always play dove) from mixed strategies based on a single, stochastically variable phenotype (e.g. every individual randomly plays hawk 40% of the time and dove 60% of the time; Bergstrom & Godfrey-Smith 1998; Orzack & Hines 2005). Similarly, evolutionary biologists studying bet hedging begin by assuming that genotypes differ with respect to the variability of their phenotypes (Seger & Brockmann 1987; Simons & Johnston 1997; Donaldson-Matasci et al. 2008), and then consider two ways that a single genotype might generate high phenotypic variability: one in which the genotype generates several different phenotypes, each of which is fixed within individuals, and the other, called 'adaptive coin flipping', in which the individuals with a given genotype stochastically express different trait values at different times (reviewed in Childs et al. 2010). Hence, theory tells us that there are at least two ways that a genotype might generate variable behaviour. One, 'behavioural polyphenism', occurs when a genotype produces several different types of individuals, each of which consistently expresses a single type of behaviour. The other, 'stochastic variability', occurs when a genotype produces a single type of individual whose behaviour varies randomly (or pseudorandomly) over time.

The behavioural polyphenism and stochastic variability options are illustrated in Fig. 1 for two genotypes, A and B, for a situation in which animals are able to choose one of four possible items (I–IV). Genotype A (behavioural polyphenism) produces four types of individuals, each of which consistently chooses a different type of item (e.g. individual 1 always chooses I, individual 4 always chooses II, and so on). Genotype B (stochastic variability) produces only one type of individual, which chooses each of the four types of items 25% of the time. When choices are aggregated across individuals within genotypes, behavioural variability is identical for genotype A and genotype B: in each genotype, each of the four items is chosen 25% of the time (see bars at the far right in Fig. 1a, b).

Thus, given evidence that IGV for behaviour does differ across genotypes from the same population, the key question is whether and how genotypic differences in variability at the individual level contribute to genotypic differences in IGV. The current study of microhabitat use in *D. melanogaster* addresses this question. In our experiment, individual flies from 14 natural genotypes from the same population were allowed free access to four different types of microhabitat, and each fly's choice of perch site was recorded on five occasions over the course of a day. Because each individual had access to four different types of habitat, we used Shannon entropy to quantify the variability of choice for each genotype, and for each of the individuals within each genotype. We first established that IGV, as measured by Shannon entropy H (Shannon 1948; Shannon & Weaver 1963) varied across the genotypes. We then used indices of entropy for each of the genotypes and indices of the mean entropy for the individuals with each genotype to test four hypotheses about different ways that within-individual variability might contribute to genotypic differences in the IGV of behaviour in this and other species.

METHODS

Flies

The genotypes were recurrent F_1s made by repeatedly crossing the same inbred parental lines, originally derived from a population in Raleigh, NC, U.S.A. The parental inbred lines are part of the *Drosophila* Population Genomics Project (DPGP.org). The direction of the crosses (i.e. maternal and paternal genotypes) was consistent, to control for maternal effects. For example, genotype A/B would be generated by crossing virgin females of genotype A to males of genotype B. The fly crosses were: 303×313, 208×712, 360×335, 639×517, 707×765, 732×775, 304×862, 306×391, 315×365, 357×714, 375×427, 437×324, 486×380 and 786×820. This experiment focused on the microhabitats used by focal males of these 14 genotypes; stimulus males and females from one genotype (303×313) were used to see whether space use patterns of the focal males changed as a function of social context.

With the exceptions indicated below, methods were the same as those described in detail in (Saltz 2011). Flies were housed and experiments conducted in an experimental room with a 12:12 h light:dark cycle, approximately 26 °C and 98% humidity. Flies were reared on standard fly food (approximately 10 ml/vial) in vials founded by 10 males and 10 virgin females to ensure that all of the larvae were reared at low (noncompetitive) densities. Within 8 h of eclosion, focal males and stimulus males were housed individually in vials; stimulus females were housed in groups of five and mated on their first day of life to a standard genotype (genotype 852 from